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A PHYLOGENETIC STUDY OF THE CHRYSOBALANACEAE USING MORPHOLOGICAL DATA

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ABSTRACT

Phylogenetic analyses were performed using morphological data to determine the monophyly and the generic relationships of the family Chrysobalanaceae. The resulting tree strongly supports the monophyly of the family. The tribal grouping of Prance and White were not recovered. The genus *Licania* is paraphyletic. The results also indicate the heterogenetic nature of the genus *Magnistipula*.

Key words
Chrysobalanaceae, phylogeny, morphological data

INTRODUCTION

The family Chrysobalanaceae R. Br. includes 17 genera and about 500 species, most of which are concentrated in the lowlands of the tropics and subtropics. The Neotropics are overwhelmingly richer in numbers of species (~ 375 species), whilst the Old World tropics (~50 species) show more morphological diversity. The present family circumscription (Prance and White, 1988) places the 17 genera in four tribes Chrysobalaneae: *Chrysobalanus, Grangeria, Licania, Parastemon*; Couepieae: *Acioa, Couepia, Maranthes*; Parinarieae: *Bafodeya, Exellodendron, Hunga, Neocarya, Parinari* and Hirtelleae: *Atuna, Dactyladenia, Hirtella, Kostermanthus, Magnistipula.*

The Chrysobalanaceae comprise a group of woody plants. Several species, including *Atuna, Kostermanthus, Licania, Magnistipula, Maranthes* and *Parinari* are trees, which exceed a height of 30m and are significant members of the upper canopy or the emergent layer of tropical rain forests. Most species are erect, but a few are scandent but lack special adaptations for climbing. Despite the wide range in height, the vegetative architecture of the plants is relatively uniform. The flower, by contrast, is comparatively diverse, although nearly every genus is characterized by an underlying uniformity of inflorescence and floral structure. Prance and White believed that this diversity is clearly related to pollen transfer (Prance and White, 1988).

The preliminary attempts to apply a cladistic analysis at the family level by Prance and White (1988) were difficult due to the widespread occurrence of parallelism

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and because only a few of the many characters satisfy the requirements for inclusion in a cladistic analysis. Chappill (1992) carried out a cladistic analysis based on morphological characters to determine the level of parallelism in the family; and to see how well Prance and White's system is supported by the characters they describe. The results of the analysis support a monophyletic family, although much of the tribal groupings of Prance and White are not well supported. Therefore, an attempt is made during this study to determine the generic relationships of the Chrysobalanaceae using additional morphological and leaf anatomical data.

MATERIALS AND METHODS

The 17 genera recognized by Prance & White (1988) in the Chrysobalanaceae were coded for their morphological characters. Two more variable genera *Licania* and *Magnistipula* were scored at subgeneric level. Characters were scored from herbarium specimens based primarily on the collection at Kew. Characters were selected by reviewing previous work and searching for variations that had not been previously analysed. This information was supplemented with published observations from the literature (Prance, 1963; 1972a; 1989; Prance & White, 1988; Prance, pers. comm.).

The Family Dichapetalaceae was used as the out-group. The selection was based on a molecular phylogenetic study using *rbc*L sequences data (Chase *et al.*, 1993; Litt & Chase 1999). Characters for the Dichapetalaceae were coded using the monograph of Prance (1972b) as a guide and by the examination of herbarium specimens.

Preparation of material

Flowers: Dried herbarium material was revived by boiling in water, dissecting and observing using a dissecting microscope (WILD M 3Z with an intralus 5000 light source).

Leaf architecture: Leaf venation, the pattern made by the primary vein (mid vein), secondary, tertiary and quaternary veins, were coded by clearing leaves and observing their patterns. The method of leaf clearing was modified from Radford *et al.* (1974).

Data analysis

The data matrix consisted of a total of 50 characters (Table 1). The matrix is presented in Table 2. Three invariable characters were excluded. Of the 50 characters 35 are binary (in which 17 of them are coded as simple absence/presence) and 15 are multistate. Cladistic analyses were performed using the test version of PAUP* 4d55 for Macintosh (Phylogenetic Analysis using Parsimony, Swofford, 1998) to assure recovery of the most parsimonious tree or trees. Alternative tree topologies and resultant changes in tree lengths were explored using MacClade 3.04 (Maddison & Maddison, 1992). For all analyses, heuristic searches were performed initially under the unordered and equal weighting criteria of Fitch parsimony (Fitch, 1971) with 500 replicates, random sequence additions, tree bisection-reconnection (TBR) branch swapping, MULPARS in effect, and steepest descent on. Ten trees were held for each step. Strict consensus, 50% majority rule and Adams consensus tree were obtained and

Table 1
List of morphological characters used in the analysis

(1) **Lamina glands**: absent = 0; present = 1.
(2) **Leaf papillae**: absent = 0; present = 1.
(3) **Stomatal crypts**: absent = 0; present = 1.
(4) **Leaf trichomes**: absent = 0; present = 1.
(5) **Epidermal cells**: non-mucilagineous = 0; mucilaginous = 1.
(6) **Silica bodies in the epidermis**: absent = 0; present = 1.
(7) **Petiolar glands**: absent = 0; present = 1.
(8) **Stipules**: absent = 0; present = 1.
(9) **Bracts**: absent = 0; present = 1.
(10) **Bracts size**: small = 0; large = 1.
(11) **Bract glands**: absent = 0; present = 1.
(12) **Receptacle tube**: absent = 0; present = 1.
(13) **Sepals**: acute =0; obtuse = 1; round = 2.
(14) **Sepal shape**: equal = 0; subequal =1; unequal = 2.
(15) **Petals**: absent = 0; present =1.
(16) **Petal length**: shorter than the calyx =0; equalling the calyx =1; longer than the calyx = 2.
(17) **Receptacle hairs**: absent=0;present at throat=1; throughout the interior=2;present in mouth and interior=3
(18) **Receptacle tube length**: shorter than calyx = 0; equal to calyx = 1; longer than calyx = 2.
(19) **Floral symmetry**: actinomorphic = 0; slightly zygomorphic = 1; strongly zygomorphic = 2.
(20) **Sexual habit**: unisexual = 0; bisexual =1.
(21) **Retrose hairs in throat**: absent = 0; present = 1.
(22) **Stamen number**: 10 or less = 0; more than 11=1.
(23) **Filaments**: absent = 0; present = 1.
(24) **Fertile stamen placement**:complete or almost complete circle=0; unilaterally opposite the carpel=1
(25) **Filament length**: shorter than the calyx = 0; as long as the calyx = 1; longer than the calyx = 2.
(26) **Filament union**: free = 0; united at base = 1; united more than 1/3 its length = 2.
(27) **Filament hairs**: absent = 0; present = 1.
(28) **Filaments in bud**: not coiled = 0; undulate = 1; coiled = 1.
(29) **Staminodes**: absent = 0; present = 1.
(30) **Ovary insertion**: base of receptacle = 0; lateral = 1; mouth of receptacle tube = 2.
(31) **Ovary locules**: unilocular= 0; bilocular-with false dissepiment= 1; bilocular- true= 2; trilocular= 3
(32) **Ovary hairs**: absent = 0; present = 1.
(33) **Stigma**: deeply lobed = 0; scarcely lobed = 1.
(34) **Style hairs**: absent = 0; present = 1.
(35) **Gynobasic style**: absent = 0; present = 1.
(36) **Drupe**: fleshy = 0; dry = 1..
(37) **Epicarp**: smooth = 0; rough = 1.
(38) **Endocarp surface**: smooth = 0; rough = 1.
(39) **Endocarp interior**: glabrous = 0; hairy = 1.
(40) **Germination**: cryptocotylar = 0; phanerocotylar = 1. (Terminology: Duke (1965, 1969).
(41) **Cataphylls**: absent = 0; present = 1.
(42) **First pair of eophylls**: opposite = 0; alternate =1.
(43) **Rays**: uniseriate = 0; biseriate = 1.
(44) **Brochidodromous venation**: absent = 0; present = 1.
(45) **Primary vein size**: less than 2mm = 0; 2.1 mm or more = 1.
(46) **Secondary vein angle**: Angle 45° = 0; angle 46°- or more = 1.
(47) **Secondary veins-variation in angle divergence**:uniform=0;non-uniform type 1=1;non- uniform type 2=2
(48) **Secondary vein course**: Type 1=secondaries gradually turning up, dividing at 1/2 of its length and the division of the secondaries that join the adjacent secondaries is visible. Type2=only some secondaries divide and do not show quite such a distinct pattern - *Neocarya* type. Type 3= secondaries turn up very close to the margin and the division of the secondaries that join the adjacent secondaries is obscure. Type 1= 0;type 2=1; type 3=2.
(49) **Tertiary vein pattern**: parallel = 0; reticulate = 1.
(50) **Areoles**: Incomplete = 0; well developed = 1.

branch lengths and tree scores were calculated using ACCTRAN (accelerated transformation optimisation). The initial trees found with equal (Fitch) weights were used as the basis for successive weighting. Successive weighting was carried out using the rescaled consistency index. Reweighting was continued until the same tree length was obtained in two successive rounds. Bootstrapping and Bremer support analyses was carried out to evaluate the robustness of the clades. The bootstrap analysis employed 100 replicates of a full heuristic search, and searching was completed with both the initial equally weighted trees and the successive weighted trees. Bootstrap support was preferred over Jackknife procedure as the method is found to be unsuitable for data sets with missing data (Nandi *et al.*, 1998). For Bremer support analysis the strict consensus tree was used as the input tree, this measures the number of extra steps it takes to collapse a group in a specific cladogram (Bremer, 1988).

Successive weighting was used to "improve" the matrix; in effect this procedure optimizes the fit of the most consistent characters on the tree such that more changes are forced into the characters found to be least consistent in the initial round of analysis. The effect of such weighting has been well documented (Nandi *et al.*, 1998; Rudall *et al.*, 1998). Generally there is a reduction in the number of trees produced because the characters that change frequently are eliminated, as they are less parsimonious. It is expected that the use of successive weighting will find trees for which the Fitch lengths are longer than for the shortest trees found with Fitch parsimony. This is due to the fact that when highly homoplasious characters are down weighted, more consistent characters (those with higher relative weights) will be optimized more parsimoniously, thus forcing more changes into already highly homoplasious characters because such actions actually reduce the weighted tree length (Nandi *et al.*, 1998).

RESULTS

Heuristic search under the Fitch criterion yielded 18 most parsimonious trees (MPTs) of 219 steps. The 18 trees were in six different island profiles (Table 3). The strict consensus tree had a CI of 0.534 and RI of 0.557 (Figure 1). The majority rule tree is shown in Figure 2. Successive weighting was carried out with the strict consensus tree of the eighteen trees (Search I) and also with the trees in the six different islands (Search II). The weighting of the strict consensus tree of search I resulted in three MPTs of 74.77 steps, CI = 0.57 and RI = 0.715 (Fitch length of 221, CI = 0.529, RI = 0.548) (Figure 3). One of MPTs is shown as a phylogram in figure 4. In search II, weighting of the trees of the different islands, resulted in similar tree statistics but with different topologies. The placement of some taxa was different in certain trees and will be referred to accordingly. In search II the tree length was 74.44 or 74.84 steps, CI =0.569 and RI = 0.714 (Fitch lengths of 221 steps, CI = 0.529 and RI = 0.548). The successive weighting reduced the number of trees during both searches. The number and distribution of unambiguous synapomorphies were calculated (Figures 1 and 3). A total of five unambiguous synapomorphies are distributed on the equally weighted tree of which two support the family clade. The number of unambiguous synapomorphies that support the internal clades increased during successive weighting. A total of seven unambiguous synapomorphies supports the strict consensus of the successive weighting (Search I), of which two support the family clade and five support internal clades.

Table 2
Data Matrix for the cladistic analysis of the combined morphological data set

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Chrysobalanus	1	0	00&1		1	1	0	1	1	0	0	1	0	1	1	2	2	0	0	1	0	1	1	0	2
Grangeria	1	0	0	0	0	1	0	1	1	0	1	1	1	1	1	0	0	0	1	1	00&1		10&1		2
Licania subg. *Moquilea*	0	0	00&1		0	1	0	1	1	0	0	1	0	10&1		1	2?		0	1	0	1	1	0	2
Licania subg.*Parinariopsis*	0	00&1		1	0	1	1	1	1	1	0	1	0	1	1	1	3	2	1	1	1	1	1	0	2
Licania subg. *Licania*	0	00&1		1	0	10&1		1	1	0	0	1	0	10&1		1	2	20&1		1	0	0	10&1		0
Licania subg. *Afrolicania*	1	0	0	0	0	1	0	1	1	0	1	1	0	1	0?		2	1	0	0	0	0	1	1	0
Licania subg. *Angelesia*	1	0	0	1	0	1	0	1	1	0	0	1	0	2	1	1	3	1	0	1	1	0	10&1		0
Licania section *Leptobalanus*	1&0	00&1	0&1		0	1	0	1	1	0	0	1	0	10&1		1	2	1	0	1	00&1		1	0	2
Parastemon	1	0	0	0	0	0	0	1	1	0	0	1	0	1	1	1	3	0	0	1	0	0	10&1		0
Bafodeya	0	0	1	1	0	1	0	1	1	0	0	1	0	2	1	1	3	1	2	1	1	0	1	1	1
Exellodendron	1	0	00&1		0	1	0	1	1	0	0	1	0	1	1	1	3	2	1	1	1	0	1	1	1
Hunga	1	0	00&1		0	1	0	1	1	0	0	1	0	1	1	0	3	2	1	1	1	0	1	1	0
Neocarya	1	0	1	1	0	1	0	1	1	1	0	1	0	2	1	0	1	0	2	1	1	1	1	1	2
Parinari	1	0	1	1	1	1	1	1	1	1	0	1	0	1	10&1		3	2	1	1	1	0	1	1	0
Acioa	1	0	0	0	0	1	0	1	1	0	0	1	2	2	1	2	1	2	2	1	1	1	1	1	2
Couepia	1	00&1	0&1		0	10&1		1	1	0	0	10&2		1	1	1	3	2	1	1	1	1	10&1		2
Maranthes	1	0	00&1		0	1	0	1	1	0	0	10&2		1	1	1	0	2	1	1	0	1	1	0	2
Atuna	1	1	0	0	0	1	0	1	1	0	0	1	0	1	1	1	3	2	1	1	1	1	1	1	2
Dactyladenia	1	0	00&1		0	1	0	1	1	0	1	10&2		2	1	1	1	2	2	1	1	1	1	1	2
Hirtella	1	0	00&1		1	1	0	1	1	00&1		1	0	1	1	0	1	0	1	1	1	0	1	1	2
Kostermanthus	1	1	0	0	0	1	0	1	1	0	0	1	2	2	1	2	2	0	2	1	0	1	1	1	2
Magnistipula subg. *Magnistipula*	1	0	0	0	0	1	0	1	1	0	0	1	0	2	1	2	3	2	2	1	1	0	1	1	1
Magnistipula subg. *Pellegriniella*	1	0	0	0	0	1	0	1	1	0	0	1	0	2	1	2	1	2	2	1	1	0	1	1	0
Magnistipula subg. *Tomiella*	1	0	0	0	0	1	0	1	1	0	0	1	1	2	1	2	3	2	1	1	0	0	1	1	2
Dichapetalum	1	0	00&1		0	0	0	1	1	0	0	0	00&1		1	0?	?		0	1?		0	1	01&2	
Tapura	1	0	0	0	0	0	0	1	1	0	0	00&2		0	1	2?	?		0	1?		0	1	1	2
Stephanopodium	1	0	0	0	0	0	0	1	1	0	0	0	0	2	1	2?	?		0	1?		0	0	0	3

Table 2 cont…

	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Chrysobalanus	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1	1	0	0	0	1	1	0	1	0
Grangeria	1	0	00&1		2	0	1	1	0	1	0	0	0	1?	?		1	0	0	1	1	0	0	1	0
Licania subg. *Moquilea*	0&1	0	0	0	0	0	1	1	1	1	1	0?	?	?	?		1	0	0	0	0	0	0	1	0
Licania subg. *Parinariopsis*	0	1	0	0	1	0	1	1	1	1	1	0?	?	?	?		1	0	0	0	1	0	0	1	0
Licania subg. *Licania*	0&1	0&1	00&1		0	0	1	1	1	10&1	0&1	?	?	?	?		1	0	0	0	10&1		0	1	0
Licania subg. *Afrolicania*	0	0	0	0	0	0	0	1	0	1	1	0?	?	?	?		1	1	0	0	1	1	0	1	0
Licania subg. *Angelesia*	0	0	0	0	0	0	1	1	1	1	1	0?	?	?	?		1	0	0	0	1	0	0	1	0
Licania section *Leptobalanus*	0	0	0	0	0	0	1	1	1	1	1	0	1	1?	?	?		0	0	0	0	0	0	1	0
Parastemon	0	0	00&1		0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Bafodeya	1	0	1	1	1	1	1	1	1	1	0	1	0	1?	?	?		1	0	0	1	0	0	1	0
Exellodendron	1	0	0	1	2	1	1	1	1	1	1	0	0	1?	?	?		0	0	0	1	0	0	1	0
Hunga	0	0	0	1	1	1	1	1	1	1	1	0	0	1?	?	?		1	0	0	1	0	0	1	0
Neocarya	1	0	1	1	2	1	1	1	1	1	1	1	1?		0	0	1	1	0	0	0	2	1	1	0
Parinari	1	0	1	1	1	1	1	1	1	1	1	1	1?		0	1	1	1	0	1	1	0	2	0	0
Acioa	2	0	2	1	2	0	1	1	1	1	0	0	0	1	1?		0	1	0	1	1	2	0	1	0
Couepia	1	0	10&1		2	0	1	1	1	10&1		0	1	1	0	1	1	1	0	1	1	1	2	0	1
Maranthes	1	0	2	1	2	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0
Atuna	1	0	0	1	2	1	1	1	1	1	0	1	1	1	0?		1	1	0	0	1	0	0	1	0
Dactyladenia	2	0	2	1	2	0	1	1	1	1	0	1	1	1	0?		0	1	0	0	1	0	0	1	0
Hirtella	0	0	2	1	2	0	1	1	1	1	1	0	0	1	0	1	1	0	0	0	1	1	0	10&1	
Kostermanthus	2	0	0	1	2	0	1	1	1	1	0	1?		0?	?	?		1	0	1	00&1		0	1	0
Magnistipula subg. *Magnistipula*	1	0	0	1	2	0	1	1	1	1	1	1?		1	0	0	0	1	0	1	1	0	0	1	0
Magnistipula subg. *Pellegriniella*	1	0	0	1	2	1	1	1	1	1	1	1?	?		0?		0	1	0	0	1	0	0	1	0
Magnistipula subg. *Tomiella*	1	1	0	1	2	0	1	1	1	1	1	1?	?		0?		0	1	0	1	0	1	0	1	0
Dichapetalum	0	0	1	0	02&3		10&1		1	0	0	1?	?	?	?	?		2	0	0	1	0	0	1	0
Tapura	3	1	1	1	0	3	10&1		1	0	0	1?	?	?	?	?		2	0	0	1	0	0	1	0
Stephanopodium	3?	?		0	02&3		1	1	1	0	0	1?	?	?	?	?		2	0	0	1	0	0	1	0

Table 3
Tree island profile resulting from the Fitch analysis of the morphological data

Island	Size	First tree	Last tree	Score	First replicate	Times hit
1	1	1	1	219	1	58
2	11	2	12	219	2	242
3	2	13	14	219	3	39
4	2	15	16	219	5	68
5	1	17	17	219	10	63
6	1	18	18	219	11	29

DISCUSSION

Phylogenetic Analysis

The strict consensus of the equally weighted tree is poorly resolved (Figure 1). Broadly seven monophyletic clades can be identified. The family is resolved as a monophyletic group with good support (96% bootstrap, 5 steps Decay and two unambiguous synapomorphies). *Parastemon* is the basal taxon in the family. *Chrysobalanus* and the *Licania* subgenera form an unresolved bush, with *Licania* subg. *Moquilea* and *Licania* subg. *Leptobalanus* forming a clade while the remaining taxa form a monophyletic clade. Within this monophyletic clade most genera are unresolved except for the following genera in brackets that form a clade (((*Acioa*, *Dactyladenia*) *Kostermanthus*) subgenera of *Magnistipula*). The 50% majority rule tree is partially resolved (Figure 2). The in-group is monophyletic and the basal taxon is *Parastemon*. Apart from *Parastemon* the family is grouped into two monophyletic clades. *Chrysobalanus* and *Licania* subg. *Afrolicania* are held together in one clade and this clade occurs in thirteen trees of the eighteen trees. The rest of *Licania* are grouped together with *Licania* subg. *Moquilea* and *Licania* subg. *Leptobalanus* forming a monophyletic clade while the rest of the three subgenera are unresolved. Sister to *Licania* is *Maranthes*, which is in turn sister to the large clade with ((*Atuna* ((*Bafodeya*, *Neocarya*), (*Parinari*, *Couepia*)) (*Exellodendron* (*Hunga* (*Grangeria*, *Hirtella*) (all subgenera of *Magnistipula* (*Kostermanthus* (*Dactyladeniya*, *Acioa*)))))). The subgenera of *Magnistipula* are unresolved and come out together with the clade (*Kostermanthus* (*Dactyladenia*, *Acioa*)) in all the trees. This whole clade is present in the large clade in all the trees recovered during the Fitch search. In nine trees *Magnistipula* subg. *Magnistipula* and *Magnistipula* subg. *Tolmiella* are resolved as a monophyletic group.

An Adams consensus tree was computed to indicate the common patterns of relationship found in the six islands (Adams, 1972). Among the equally parsimonious trees, the major difference is in the relative positions of *Chrysobalanus*, *Licania* subg. *Afrolicania*, *Licania* subg. *Licania*, *Grangeria*, *Couepia*, *Hunga*, *Maranthes* and *Hirtella*.

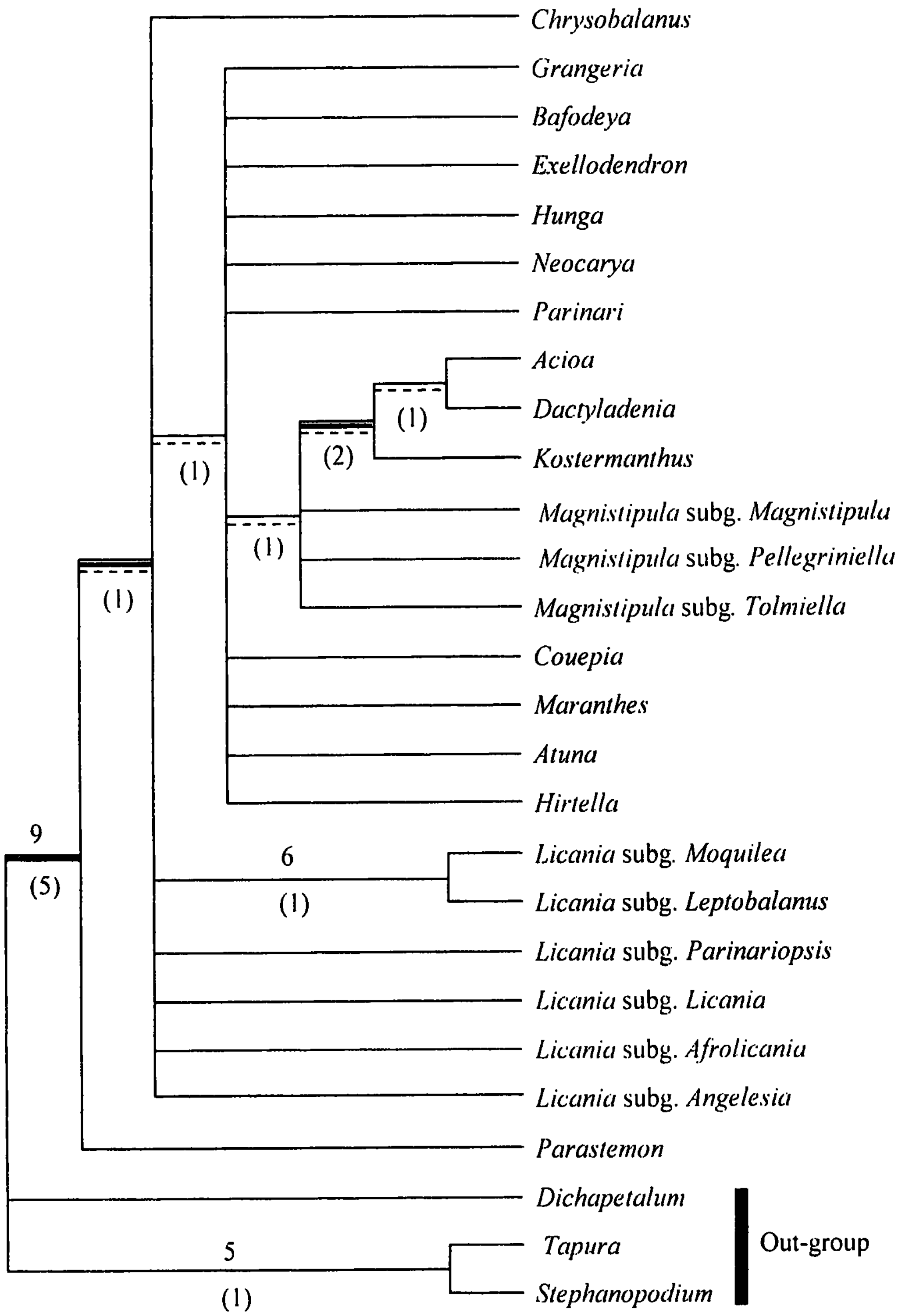

Figure: 1 Strict consensus tree of the eighteen trees recovered during the Fitch Parsimony analysis of the morphological data set. Length = 219, CI = 0.534, RI = 0.557. The bootstrap support values are shown above branches. The branches that collapse during bootstrap analysis is indicated as dotted lines. The figures in brackets below the branches are Bremer support values. The thick branches indicate the distribution of unambiguous synapomorphies.

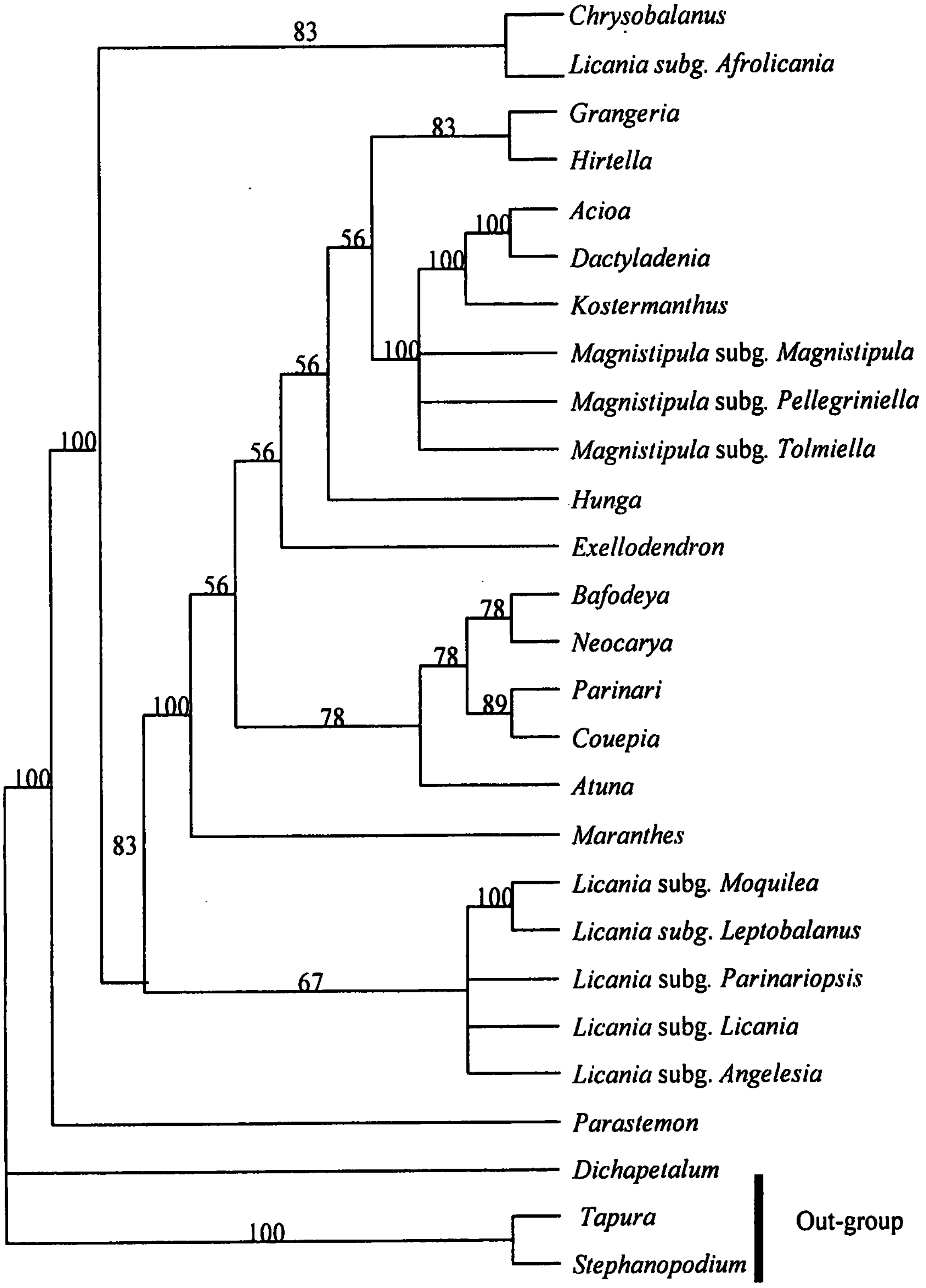

Figure: 2. 50% Majority rule tree of the eighteen trees recovered during the Fitch Parsimony analysis of the morphological data set.

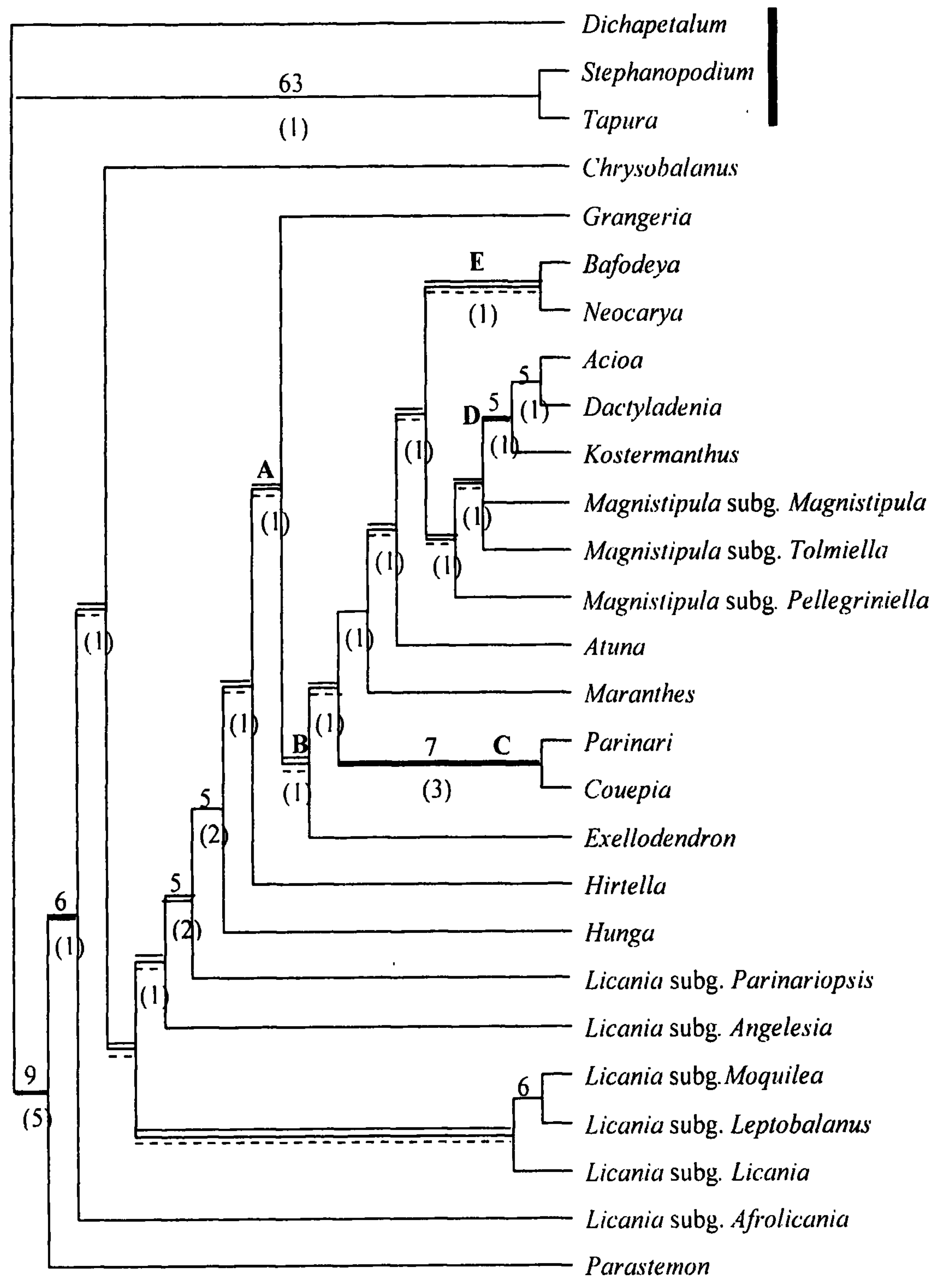

Figure: 3. Strict consensus of the three MPT recovered during the successive weighting-Search I of the morphological data set. Length = 74.77, CI = 0.57, RI = 0.715. The Bootstrap support values are shown above the branches. The branches that collapse during bootstrap analysis is indicated as dotted lines. Figures in brackets below the branches are the Bremer support values. The double lines are the branches that are not present on the Fitch tree. The thick branches Is the distribution of unambiguous synapomorphies.

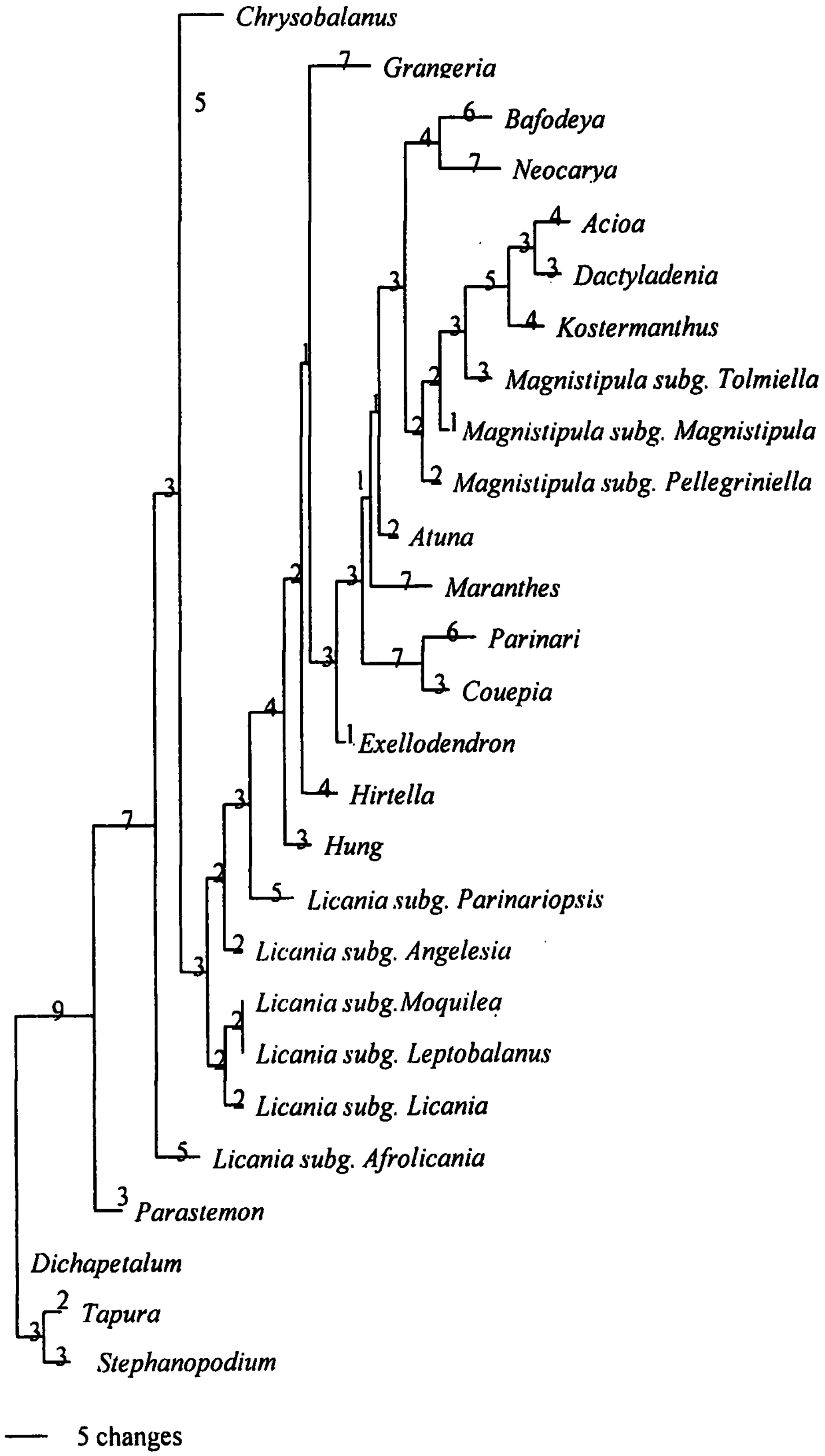

Figure: 4. One of the MPTs resulting from the successive weighting of the strict consensus of the morphological data. The figures above the branches are the Fitch lengths.

Of the outgroup taxa, *Tapura* and *Stephanopodium* form a monophyletic clade with 63% bootstrap and one step Decay while *Dichapetalum* is unresolved.

The strict consensus tree resulting from search I (Figure 3) is fully resolved except for the placement of *Magnistipula* subg. *Magnistipula* and *Magnistipula* subg. *Tolmiella.*

This tree represents all the major grouping recovered by all the trees resulting from weighting of the different six islands under search II. The tree resulting from weighting of the first, third and fourth island profiles differed from the tree resulting from search I, only in the placement of the clade bearing (*Licania* subg. *Licania* (*Licania* subg. *Moquilea*, *Licania* subg. *Leptobalanus*)). In the case of search II *Licania* subg. *Moquilea* and *Licania* subg. *Leptobalanus* come out as a separate monophyletic clade next to *Licania* subg. *Licania.* The tree that resulted from weighting of the second and sixth island profiles differed from search I tree in the placement of the Clades (*Bafodeya*, *Neocarya*), (*Parinari*, *Couepia*) and *Magnistipula.* The clade with (*Bafodeya*, *Neocarya*) is broken but the two taxa come out next to each other as separate clades. The Clade with (*Parinari*, *Couepia*) is placed in between *Atuna* and the Clade with ((*Bafodeya, Neocarya*) (*Magnistipula* subg. *Pellegriniella* (*Magnistipula* subg. *Tolmiella*, *Magnistipula* subg. *Magnistipula*) (*Kostermanthus* (*Dactyladenia*, *Acioa*)))). The unresolved *Magnistipula* subg. *Tolmiella* and *Magnistipula* subg. *Magnistipula* form a monophyletic clade on the search II tree. The tree resulting from weighting of the fifth island profile was different from the tree resulting from search I in all the above mentioned positions. Therefore, as the search I tree represents a consensus of all searches, the discussion of the relationships is mainly based on the tree recovered from search I (Figure 3).

The family is recovered as a monophyletic group (99% bootstrap, 5 steps Decay and two unambiguous synapomorphies). *Parastemon* is the basal taxon, and is sister to the rest of the family. The subgenera of *Licania* are paraphyletic with *Licania* subg. *Afrolicania* as the ancestral taxa next to *Parastemon*. *Chrysobalanus* is nested between *Licania* subg. *Afrolicania* and the clade containing (*Licania* subg. *Licania* (*Licania* subg. *Leptobalanus*, *Licania* subg. *Moquilea*). This clade as a whole is not supported but the internal clade (*Licania* subg. *Leptobalanus*, *Licania* subg. *Moquilea*) is weakly supported (65% bootstrap). Sister to this clade is a grade of taxa, *Licania* subg. *Angelesia*, *Licania* subg. *Parinariopsis*, *Hunga*, *Hirtella*, *Grangeria* and *Exellodendron* up to the grouping of *Parinari* and *Couepia*, Clade C, that contains a support value of 76% bootstrapping. Clade C is sister to larger clade containing the remaining taxa. *Maranthes* and *Atuna* are sister to a large clade consisting of two smaller sister clades. The first clade contains *Bafodeya* and *Neocarya* while the second clade contains *Magnistipula* subg. *Pellegriniella* basal and sister to unresolved *Magnistipula* subg. *Tolmiella* and *Magnistipula* subg. *Magnistipula*. These taxa are in turn basal to (*Kostermanthus* (*Acioa*, *Dactyladenia*)). The tree was broadly divided into five monophyletic clades A, B, C, D and E for convenience of comparison (Figure 3). The presence of the receptacle tube and gynobasic style are two unambiguous synapomorphies for the family clade. The presence of silica bodies in the epidermis is an unambiguous synapomorphy for the family clade except for *Parastemon*. The round sepals and ligulately connate stamens (staminal ligule) are two unambiguous synapomorphies for the Clade D (Figure 3). The character state leaf secondary vein apex Type III and parallel tertiary veins are two unambiguous synapomorphies for the Clade C. Round sepals support a monophyletic clade D. The condition obtuse sepals have evolved independently in two clades *Grangeria* and *Magnistipula* subg. *Tolmiella* within the clade acute sepals. Unequal sepals form a monophyletic clade with Clades E, D and *Magnistipula*. This condition also occurs in *Licania* subg. *Angelesia* and *Stephanopodium*.

On all trees recovered the outgroup taxa *Tapura* and *Stephanopodium* are monophyletic while the placement of *Dichapetalum* is unresolved (63% bootstrap and one step Decay).

Phylogenetic Implications

The family is monophyletic but the resulting trees from all searches do not support the tribes of the family of the Prance and White's most recent classification (1988). In the tribe Chrysobalaneae *Parastemon, Chrysobalanus* and *Licania* (all sub-genera) are placed sister to each other except for *Grangeria* which is placed as a separate clade within Clade A. In the tribe Hirtelleae *Dactyladenia, Kostermanthus* and *Magnistipula* (all sub-genera) are group together with *Acioa* of the Couepieae while *Atuna* and *Hirtella* are scattered among the other genera. The taxa within tribes Couepieae and Parinarieae are integrated among other genera. The tree topology needed 15 homoplasious steps to reach the Prance and White classification.

Licania are paraphyletic. Out of the eighteen trees recovered during the Fitch search fifteen trees found *Licania* subg. *Afrolicania* grouped with *Chrysobalanus* on a clade, which is separated from the rest of *Licania*. The other three trees place it within the rest of *Licania*. Going through the characters *Licania* subg. *Afrolicania* is separated from the rest of *Licania* by five characters. Being unisexual and the absence of hairs on the ovary and style make this taxon different from the rest of the family as well. Even though *Licania* subg. *Afrolicania* is placed with *Chrysobalanus* on most of the equally weighted trees and is separated from the rest of *Licania* also by *Chrysobalanus*, it does not share any remarkable character combinations with *Chrysobalanus*. Considering the patristic distance matrix (not given) *Licania* subg. *Afrolicania* is more close to *Chrysobalanus* than to the rest of *Licania*. Therefore the phylogenetic analysis of the morphological data suggests a separate position from the rest of *Licania* for *Licania* subg. *Afrolicania*, supporting the resurrection of the genus *Afrolicania* (Yakandawala *et al.*, submitted).

Licania subg. *Moquilea* section *Leptobalanus* with unique character combinations, was scored as terminal taxon subgenus; *Licania* subg. *Leptobalanus* forms a monophyletic group with *Licania* subg *Moquilea*. There is no length difference on the phylogram between these two taxa. Only this clade together with *Licania* subg. *Licania* is monophyletic. *Licania* subg. *Parinariopsis* with the laterally placed ovary is once again another odd group within *Licania*. It also differs from the rest of the *Licania* by the presence of two large alternative glands on the petiole, the presence of large bracts and bracteoles and the presence of hairs in the filaments. This taxon is considered to be more related to *Licania* subg. *Moquilea* (Prance, 1972a), but the results indicate it is more related to *Licania* subg. *Angelesia* and is also sister to the rest of the family clade. Therefore the placement of *Licania* subg. *Parinariopsis* within *Licania* is also a point of debate. Even though *Licania* subg. *Angelesia* comes out on a separate clade there are no strong morphological features that separate it from *Licania*. *Magnistipula* are shown to be paraphyletic (Figure 3). *Magnistipula* subg. *Magnistipula* and *Magnistipula* subg. *Tolmiella* are unresolved on the successively weighted tree. *Magnistipula* subg. *Pellegriniella* is placed as sister to this clade and Clade (*Kostermanthus* (*Acioa*, *Dactyladenia*)). These clades have no bootstrap or decay value support. On both the equally weighted and 50% majority rule tree *Magnistipula* is an unresolved bush. In half of the equally weighted trees *Magnistipula* subg. *Magnistipula* and *Magnistipula* subg. *Tolmiella* are resolved as a monophyletic clade. *Magnistipula* subg. *Pellegriniella* is placed as sister to this clade and the monophyletic clade (*Kostermanthus* (*Dactyladenia*, *Acioa*)). The separation of *Magnistipula* subg. *Pellegriniella* is primarily because of the ovary is bi-locular. This taxon was originally described under *Parinari senu lato* for the reason of sharing the spurious dissepiment but as it differs in most features it was removed from *Parinari senu lato* together with the others. As its flower was virtually indistinguishable from those of some species of *Magnistipula* except for the dissepiment, Prance and White placed it under *Magnistipula*. *Magnistipula* subg. *Tolmiella* differs from the rest primarily by the flower being slightly zygomorphic and absence of retrose hairs. Going through the rest of the features it is apparent that the genus *Magnistipula* is somewhat variable in floral and fruit characters. The variation of floral characters, however, although the extremes are so different, forms a continuous series. Variation in fruit characters is independent of variation in other features. Prance and White (1988) are "happy" in treating the genus by splitting the genus into three subgenera, one of which is further divided into three sections. According to them splitting the genus would entail the recognition of 6 or 7 genera for 11 species, which will lead to very unsatisfactory situations. Considering the patristic distance matrix, the three taxa do not show a remarkable distance between them. However, the resulting cladogram clearly indicates the heterogenetic nature of the genus.

The placement of the ovary and floral symmetry are two characters that have been used during the grouping of the family. The basal position of the ovary is the plesiomorphic state while the ovary attachment to the mouth of the receptacle tube is advanced. In *Parinari* and *Bafodeya* the state of lateral attachment of the ovary appears to be a reversal. Similarly, in floral symmetry, the strongly zygomorphic flower is the advanced state. Once again in *Magnistipula* subg. *Tolmiella* the slightly zygomorphic condition appears to be a reversal. By looking at the morphological data set and from the recovered trees it is evident that very few characters support the monophyletic clades as unambiguous synapomorphies. According to Farris (1983), cladistic analysis provides a very powerful method of extracting the signal from noise, in a data set showing substantial homoplasy. If there are many conflicts between characters these will be revealed as unresolved regions on the consensus of the minimal length cladograms. If the parallelism were so common that no branching patterns can be retrieved from the data then the result would be a polytomous bush. The tree recovered during the initial Fitch search resulted in several unresolved bushes. Once the homoplasious characters were down weighted during successive weighting the resulting tree was fully resolved except for *Magnistipula*. Most of the groupings have very poor support. This is mainly due to the wide occurrence of parallelism in most of its characters used in the data set. This wide occurrence of parallelism has also been explained in detail as the "principle of discordant characters" during the monographing of the family (Prance and White, 1988). It is not surprising that the tribal groupings were not been recovered during the analysis as the monograph states, "we are therefore unable to offer any very definite views on the phylogenetic relationships of the genera. For convenience, we have grouped them into four tribes and we believe that this treatment may partly reflect a major pattern of divergence within the family, but we are not sure that we have correctly placed all genera, and are unable to suggest convincing branching patterns within tribes". Furthermore, in addition to traditional characters they also relied on character syndromes. These character syndromes "even though they are individually small and rarely absolutely diagnostic, are extremely well correlated and collectively provide highly. diagnostic criteria for generic delimitation". "The syndromes are functional and are of vital importance to the plant. They determine, for instance, the precise way in which nectar and stigmatic and polliniferous surfaces are presented, both in space and time, to potential vectors, and the means of seed protection and dispersal, and of seedling release from the endocarp". Therefore another reason for not recovering any tribal groupings could be the fact that some of these characters that have been perceived 'by eye' have not been included in the delimition of taxa and therefore were not included in the analysis. The trees recovered during Chappill's study (1992) were not in congruent with these trees, except that *Chrysobalanus* and *Licania* come together. Her suggestion of uniting *Licania* with *Chrysobalanus* is not acceptable and the reasons will be discussed elsewhere (Yakandawala *et al.*, unpublished).

The great strength of cladistic analysis is that it produces hypotheses of relationships that reduce conflicts among the data to a minimum and gives an objective measure of the level of those conflicts among the data. If low levels of parallelism were a prerequisite for cladistic analysis, the method would have little application since moderate to high levels are the rule, not the exception, in most plant and animal groups (Sanderson and Donoghue, 1989). The resulting cladogram from successively weighting best describes the relationships with the available morphological characters for the family. Due to the low support it is very unlikely that this could be claimed as reflecting the true phylogeny. This may be because the characters described are those that allow ready identification of herbarium material. This stresses the need for more phylogenetically informative characters from other fields, such as molecular biology and phytochemistry.

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